MARKED-UP VERSION

In the example, the method of the invention first involves identifying an mRNA target sequence and then forming forward and reverse primer nucleotide sequences coordinated with the target sequence. This is ordinarily accomplished by a software program such as GenBank-

Entrez. The target sequence of the cDNA derived from the mRNA is as follows: [AGCACCTTCGGGATGAATCAGGCAACAGCTACTCTCCTG] SEQ ID NO: 1 agcaccttcg ggatgaatca ggcaacagct actctcctg. The forward primer for the cDNA amplification is an oligodeoxynucleotide having a nucleotide sequence: [5' –AGCACCTTCGGGATGAATC-3'] SEQ ID NO: 2 agcaccttcg ggatgaatc. The reverse primer for use with cDNA amplification is an oligodeoxynucleotide having the following nucleotide sequence: [5'-CAGGAGAGTAGCTGTTGCC-3'] SEQ ID NO: 3 caggagagta gctgttgcc. The distance between the nucleotide binding sites on the target sequence is one nucleotide. The fluorescent dyes applied to label the primers are Oregon Green 488 for the forward primer and Alexa 633 for the reverse primer, both dyes having been obtained from Molecular Probes, Inc., of Eugene, Oregon. The synthesis of the primers includes standard phosphoramidate solid phase synthesis including use of an amino-modifier – deoxyribocytidine (dC) – CPG at the 3' end of the primer. The Oregon Green 488 and Alexa 633 dyes, in the form of succynilimide (NHS) esters, are attached to the forward and reverse primers, respectively, at the primary amino group at the 3' end deoxyribocytidines (dC's) by NHS-primary amino conjugation, according to the manufacturer's protocols. The 3' OH groups are left free, and are the starting points for polymerization. The resulting labeled primers are purified by reverse phase high pressure liquid chromatography (RP/HPLC) using a TEAA-acetonitrile solvent system. The primers are then lyophilized, resuspended in deionized water, and the solution divided into 10 microliter doses having a primer concentration in solution of about 5 micromoles per liter.

The monitoring of fluorescence emission during PCR and reverse transcription thermal cycling were both performed in the LighCycler™ (Roche). The reaction vessel for both the reverse transcription step and polymerase chain reaction is a capped, optically transparent